REMARKS

Claims 45-78 are all the claims pending in the application. New claim 45 corresponds to claim 1 but recites that the cell exhibits controlled expression of recombinase Cre. The claim further recites that the control is in a FLP-dependent manner in the presence of recombinase FLP, which is provided by a recombinant adenovirus. Finally, the claim recites that the cell is a host cell for producing a recombinant adenovirus vector expressing a desired protein together with a helper virus.

Support for new claim 45 is found throughout the specification, such as in Example 1, beginning at page 26. Support for the language that the cell exhibits controlled expression of recombinase Cre is found at page 4, lines 9-15. Claims 46-78 parallel claims 2-12 and 21-42 in the amendment filed April 21, 2003, except that claims 52, 53, 61, 62, 66 and 67 have been amended to properly recite a drug "resistance" gene, which is a gene that confers resistance to a drug, as opposed to a gene that is resistant to a drug as previously recited.

No question of new matter arises and entry of the amendments is requested, respectfully.

Office Action Summary

Disposition of Claims

Claims 1-44 have been canceled. New claims 45-78 have been added.

Information Disclosure Statement

The examiner returned a copy of PTO 1449 accompanying the Information Disclosure Statement filed on November 26, 2002. However, one of the references was not initialed, and no reason was given for not initialing that reference. Further, the examiner has not returned an

initialed copy of the PTO Form 1449 that accompanied the Information Disclosure Statement filed on April 11, 2001.

In the next communication, the examiner is respectfully requested to return initialed and signed copies of PTO Forms 1449 filed November 26, 2002 and April 11, 2001.

Detailed Action

Claim Rejections - 35 U.S.C. § 103

The rejection of claims 1-12 and 21-42 under 35 U.S.C. § 103(a) as being unpatentable over Hardy (WO 97/32481) and Wahl et al. (WO 92/15694) was maintained.

In response to this rejection in the previous Office Action, Applicants pointed out that Hardy does not teach expression of Cre in a controlled manner, and that Wahl does not teach expression of Cre. Accordingly, applicants explained that there is no motivation to combine the references. In addition, Applicants pointed out that the invention is extremely useful in a helper-dependent adenovirus vector system.

The Examiner replied that the intended use, namely in a helper dependent adenovirus vector system, is not a limitation of the claims, and therefore, is not considered in determining patentability. The Examiner further appeared to take the position that the discussion in Hardy, stating that it is not advantageous to grow recombinant virus in a cell expressing Cre all the time, is sufficient motivation to look for a way to construct a cell that expresses Cre in a controlled manner.

For the following reasons, this rejection is respectfully traversed.

1. Present Invention

As seen from claim 45, the present invention relates to a cell that exhibits controlled expression recombinase Cre in the presence of recombinase FLP in a FLP-dependent manner.

More specifically, as recited in claims 46 to 49, the present invention also relates to a cell derived from a 293 cell that expresses Cre in a FLP-dependent manner and that expresses adenovirus E1A necessary for the replication of an adenovirus. The claimed cell is novel and unobvious particularly in that Cre is not permanently expressed, but rather it is transiently expressed when desired and when the adenovirus E1A gene is expressed. Thus, the claimed cell can remarkably reduce the cytotoxicity of Cre against the cell, and advantageously express Cre at a high level only when Cre is needed.

The claimed cell is extremely useful in a helper-dependent adenovirus vector system for constructing an adenovirus vector using a helper virus, as discussed below in more detail.

Specifically, the specification describes in detail, at page 10, line 25 to page 12, line 21, the construction of 293FNCre cells which are a preferred embodiment of the present invention. The 293FNCre cells have been constructed by introducing a CAG promoter, a recognition sequence of FLP (FRT), a stuffer sequence, a second FRT and the Cre gene, in this order from upstream, into the genome of 293 cells expressing adenovirus E1A, as recited in claims 48 and 49. The 293FNCre cells of the present invention can express Cre at a high level transiently in a FLP-dependent manner when Cre is needed.

Thus, the 293FNCre cells are extremely useful in a helper-dependent adenovirus vector system for constructing an adenovirus vector for use in transfecting a foreign gene, using a helper virus.

As described in detail in the specification at page 12, line 22 to page 14, line 4, an adenovirus vector is constructed by a helper-dependent adenovirus vector system using 293FNCre cells together with a helper virus.

That is, an adenovirus vector having a foreign gene, but having only inverted terminal repeats (ITR) and packaging sequences and thus incapable of replicating by itself, is transfected into the 293FNCre cells. Simultaneously, a helper virus having a packaging sequence flanked by loxP sequences and capable of providing all functions necessary for the replication of the adenovirus vector having the foreign gene is also transfected into the 293FNCre cells.

In the 293FNCre cells, when introducing FLP protein or FLP gene into the cells, Cre is transiently produced at a high level in a FLP-dependent manner. The thus produced Cre excises the packaging sequence from the helper virus to render the helper virus itself unpackagable. As a result, the propagation of the helper virus is inhibited. However, because the helper virus can provide all functions necessary for the replication of the adenovirus vector having the foreign gene, the titer of the desired adenovirus vector having the foreign gene can be increased in the 293FNCre cells.

Consequently, from the 293FNCre cells, the objective adenovirus vector having the foreign gene is obtained at a high level, while the undesired contamination consisting of the helper virus is maintained at a quite low level.

Therefore, the 293FNCre cells are extremely useful in a helper-dependent adenovirus vector system for constructing an adenovirus vector for transfecting a foreign gene, using a helper virus.

Furthermore, as described in the specification at page 14, lines 1 to 4, in the method as discussed above when introducing FLP protein or FLP gene into the 293FNCre cells, an adenovirus vector is much more preferably used which expresses FLP protein and has a packaging sequence flanked by loxP sequences. This is because such an adenovirus vector has both of the functions of (1) an adenovirus vector expressing FLP protein in the 293FNCre cells thereby to express Cre in a FLP-dependent manner and (2) also a helper virus having a packaging sequence flanked by loxP sequences thereby to be inhibited in propagation but to provide all functions necessary for the replication of the adenovirus vector having the foreign gene.

In other words, the 293FNCre cells according to the present invention enable an extremely excellent helper-dependent adenovirus vector system for constructing an adenovirus vector for transfecting a foreign gene, using a helper virus.

The novelty of the claimed cell is particularly in the expression of Cre not constantly but transiently when desired, thereby to reduce the cytotoxicity of Cre, and is thus also extremely useful in a helper-dependent adenovirus vector system as discussed above in detail.

2. Applicants' Comments

In the Final Office Action, based on the discussion at page 14, lines 26 to 31 of Hardy, the examiner asserted that "Hardy discloses that it is not advantageous to grow recombinant virus in a cell expressing Cre all the time."

Consequently, the examiner asserted as follows:

"One of skill in the art would know that gene expression can be regulated. In order to control Cre expression, one of skill in the art would be able to search for methods of regulation

of gene expression and would find the system of *Wahl* and be motivated to combine them because Wahl teaches that the FLP recombinase can be used to regulate gene expression."

However, for the reasons set forth below, there is no motivation whatsoever in *Hardy* to combine *Hardy's* teachings with the teachings of *Wahl*.

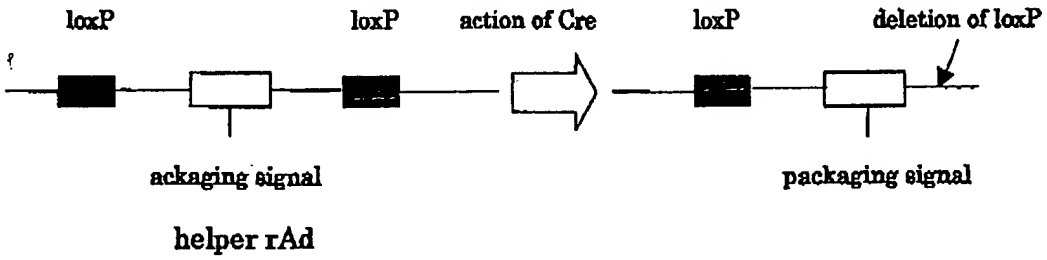
i) Hardy states at page 14, lines 29 to 31 as follows:

> ".... Non-Cre expressing cells are preferred because of the possibility that prolonged selective pressure will result in unwanted deletion in the helper rAd vector."

With respect to "unwanted deletion in the helper rAd vector," *Hardy* states at page 27, lines 20 to 23 as follows:

> "By limiting passage of the helper rAd through Cre-expressing host cells to the final infection step, helper virus is not under continuous selection and the possibility of mutations rendering the helper resistant to Cre selection is lessened."

Thus, the "unwanted deletion" is the mutations rendering the helper resistant to Cre selection. More specifically, the "unwanted deletion" in *Hardy* is the deletion of either of two Cre recognition sequences (*i.e.*, loxP) in the helper rAd as shown below:



When the deletion of either of two loxPs occurs, the action of Cre is no longer able to cut off the packaging signal located between the two loxPs, resulting in that the helper rAd becomes resistant to Cre. Therefore, even though the Cre expressing cells are used in the final step, not only the therapeutic rAd but also the helper rAd grow, so that only the therapeutic rAd is not selectively propagated, and the intended object can not be attained.

That is, *Hardy's* concept is that the Cre expressing cells are used only in the final step requiring the cutting off of the packaging signal between two loxPs in the helper rAd. *Hardy* does this in order to avoid the situation that occurs, where the Cre expressing cells are used all the time, namely that the pressure of the continuously expressed Cre results in the continuous deletion of loxP, which renders the system unable to cut off the packaging signal. (Please note that, although it is not known whether the loxP is actually deleted due to the pressure of Cre, *Hardy* appears to consider that loxP is deleted in such a way.)

Hence, *Hardy's* concern is with the harmful effect of Cre on the helper rAd. *Hardy* is not concerned with the harmful effect of Cre on the Cre expressing cell *per se*, with which the present invention is concerned.

ii) Table I, attached hereto, compares *Hardy's* concept with the concept of the present invention.

As seen from Table I, the problem *Hardy* solves is quite different from the problem the present invention solves.

Hardy's problem is solved simply by using a Cre expressing cell only in the final step.

On the other hand, the problem to be solved with the present invention is to reduce the harmful effect of Cre on the Cre expressing cell for constructing an recombinant adenovirus

vector together with a helper virus. The problem of the present invention is solved by using the new host cell that exhibits controlled expression of Cre in a FLP-dependent manner at a high level transiently only when Cre is needed, as claimed in new claim 45.

Thus, the problem of the present invention can not be solved by the solution of *Hardy* that the Cre expressing cell is used only in the final step.

As is clear from *Hardy*, the Cre expressing cell to be used in *Hardy* may be a conventional cell expressing Cre. Thus, the Cre expressing cells conventionally used in the art can be used in *Hardy* to solve the problem of *Hardy*. Therefore, there is no motivation whatsoever in *Hardy* for a person skilled in the art to look to *Wahl* to modify the cell.

Furthermore, even if a person skilled in the art wanted to combine *Hardy* with *Wahl,* he would be unable to know how to combine them with each other, because Hardy uses the conventional Cre expressing cells, whereas *Wahl* uses the specific cells expressing a gene in a FLP-dependent manner.

Indeed, before the present application was filed, the other famous researchers had also used the conventional Cre expressing cells for constructing a therapeutic rAd by cutting off a packaging signal in a helper virus due to the action of Cre, similarly to *Hardy*. In this regard, applicants submit herewith copies of two articles, Lieber A., et al., J. Virol., Vol. 70, p. 8944-8960, 1996; and Parks RJ., et al., Proc. Natl. Acad. Sci., Vol 93, p. 13565-13570.

In view of the foregoing, applicants respectfully submit that there is no motivation whatsoever in *Wahl* as well as *Hardy* to combine *Hardy* and *Wahl.*

Therefore, the examiner's rejection is made in hindsight by reference to applicants' specification.

Consequently, the claimed invention is be unobvious over *Hardy* in combination with *Wahl*.

In view of the above, reconsideration and allowance of this application are now believed to be in order, and such actions are hereby solicited. If any points remain in issue which the Examiner feels may be best resolved through a personal or telephone interview, the Examiner is kindly requested to contact the undersigned at the telephone number listed below.

The USPTO is directed and authorized to charge all required fees, except for the Issue Fee and the Publication Fee, to Deposit Account No. 19-4880. Please also credit any overpayments to said Deposit Account.

Respectfully submitted,

Jennifer M. Hayes
for Reg No. 40,641

Susan J. Mack
Registration No. 30,951

SUGHRUE MION, PLLC
Telephone: (202) 293-7060
Facsimile: (202) 293-7860

WASHINGTON OFFICE
23373
CUSTOMER NUMBER

Date: December 29, 2003